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Bacteria Isolated from Fish Pond Water and Sediment in Selected Fish Pond Ecosystems in the Osun State: Multidrug Resistance Profiles

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Abstract

Background: Globally, resistant bacteria are proliferating, and aquatic environments are quickly turning become reservoirs for resistant microorganisms. This study was aimed primarily at discovering and wanting to describe MDR bacteria in fish pond environments from Osun State, Nigeria. **Methodology**: Purposive sampling was employed in this study to collect 60 sediment and 60 fish water samples from fish ponds throughout the state. The bacterial isolates were identified by normal microbiological procedures, and the antibiotic sensitivity to twelve of the most regularly used antibiotics was assessed using the disc diffusion method. **Results:** *E. coli, Salmonella spp., Klebsiella spp., Pseudomonas aeruginosa, and Staphylococcus aureus* were among the microorganisms found. There was also substantial resistance against second-generation ciprofloxacin, vancomycin, cefuroxime, ceftazidime, cefotaxime, and gentamicin. In particular, there were several strains that produced extended-spectrum beta-lactamases (ESBLs); *Klebsiella species* and *E. coli* were shown to be resistant to several antibiotic classes. **Conclusion**: This study highlights the significance of efficient surveillance and control strategies to address the issue of resistance while identifying the rising occurrences of MDR bacteria in fish pond ecosystems. Appropriate use of antibiotics in aquaculture farming convention is a key policy in the conservation of water bodies and human wellness.

Keywords: Antibiotic resistance, bacteria, fish ponds, isolation, characterization, aquaculture

Introduction

As an important subsector of aquaculture, fish farming has recently grown rapidly worldwide (Golub & Varma 2014). In as much as the interest in this sector of fish farming is gradually increasing in the country, the outbreak of infectious diseases is a significant challenge to fish farming management practices, leading to year-round economic loss (Pridgeon & Klesius, 2012).

Antibiotic resistance is one of the greatest global health and environmental challenges, with aquaculture systems increasingly recognized as critical reservoirs both antibiotic-resistant genes (ARGs) and antibiotic-resistant bacteria (ARBs) (World Health Organization [WHO], 2020; Food and Agriculture Organization [FAO], 2020). Antibiotics are extensively utilized to avert and address bacterial illnesses. In China, the total production of antibiotics has kept increasing and was up to 223,000 tons in 2020, of which half was used for animal farming and the other half for human medical care (https://www.chinabgao.com/k/kangshengsu/66064.html accessed on 25 August 2024). Antibiotic-resistant microorganisms can emerge after the long-term use of antibiotics, which can enter aquatic systems, such as rivers and lakes, causing selective pressure on bacteria and leading to the enrichment of ARB and the evolution of ARGs (Muniesa et al., 2013; Hu et al., 2018; Su et al., 2018; Xiang et al., 2018; Su et al., 2019). By 2020, the antibiotic resistance in aquacultural systems in China had exceeded 50% (https://m.yicai.com/news/ 101235766.html accessed on 18 August 2024). The above points then established that an aquaculture environment is ideal for acquiring data about ARB and ARGs. Given the economic importance of the aquaculture industry, research interest in this area has increased (Taylor et al., 2011; Marti et al., 2014). High-density farming, overfeeding, climate change, and human activity have all led to disease outbreaks (Mao et al., 2020), and there has been an increasing trend in bacterial antibiotic resistance in the aquacultural industry. Wu et al. (2019) found that the abundance of ARGs during the fishing period was significantly higher than that during the rearing period in aquaculture cages in both Guangdong and Hainan. Yuan et al. (2019) reported increasing ARGs with increased rearing density and pond age in aquacultural ponds in Hangzhou Bay. Therefore, investigating antibiotic resistance profiles in breeding ponds during the early breeding stage can guide the proper use of antibiotics during the breeding season, thus avoiding the overuse of antibiotics and the accumulation of antibiotic resistance. It has also been reported that bacteria in the breeding ponds, such as *Acinetobacter spp*. and *Aeromonas spp*., usually contain a large number of ARGs and are often the main pathogenic bacteria during the breeding process, causing surface bleeding, rotten tails, and visceral damage to fish (Ye et al., 2021). The highest levels of antibiotic resistance in these pathogens were found against penicillin, macrolides, sulfonamides, and tetracyclines (Gai et al., 2016). The continuous and indiscriminate use of these antibiotics has resulted in requiring high doses for effective control that have put selective pressure on resistant bacteria (Agola et al., 2017). Therefore, identifying ARB is conducive to the antibiotic control of specific bacterial pathogens. The primary objective of this study was to isolate and characterize multidrug-resistant bacteria profiles from fish pond ecosystems, focusing on water and sediment samples.

Materials and Methods

The study location

The study was conducted in Osun State, in Nigeria’s South-western geopolitical zone. It covers a total land area of about 14,875 km^2. It is bounded in the East by Ondo and Ekiti States, in the West by Oyo State, while Kwara and Ogun States are its boundaries in the North and the South (Osun State Government diary, 2005). Federal Office of Statistics (FOS) (2007) reported that Osun State has a population of 3,423,535 people as of the 2006 National Census. Osun State has thirty Local Government Areas (LGAs) with several towns, villages and settlements. Osun State housed the Yoruba ethnic group, which included some dwellers from other parts of the country and even outside the country. The southern region of Osun State is characterized as a rainforest zone, exhibiting a mean annual precipitation of 1420 mm; the North is a derived Savanna with a mean annual rainfall of 1133 mm. The primary occupation of the people in Osun State is farming, as is obvious in its numerous villages. The climatic condition of Osun State favours the growth of varieties of plants, including arable and permanent crops such as locust beans, maize, cassava, groundnut, sweet potatoes, palm trees, cocoa trees, etc. Also, it accommodates rearing of livestock and fishery products (Figure 1).

Study design and sample collection

Sample collection for laboratory analysis

The study was conducted in small-to-medium-sized fish farms actively producing fish, purposively selected across Osun ’State’s three main senatorial districts (Osun Central, Osun East, and Osun West). Two LGAs were chosen from each senatorial district, designated A to F. Each pond had an average stocking density of 5–10 fingerlings/m², with surface areas of 6–20 m² for concrete tanks, 2.16 m² for plastic tanks, and 500 m² for earthen ponds. A total of 60 water samples and 60 sediment samples were aseptically collected from fish ponds.

Sampling took place from December 2022 to March 2023, during the hours of 9:00 AM to 12:00 PM. Samples of water were gathered at a depth of 30 cm. below the water surface, while sediment samples were taken at a depth of 60 cm below the pond floor. Sterile, labeled screw-cap sample bottles were used for collection to prevent contamination. Composite samples were obtained from concrete ponds, earthen ponds, and plastic ponds. Once collected, the samples were placed in a cooler containing ice pack to maintain temperatures and minimize microbial growth during transport to the laboratory. Upon arrival at the laboratory, the samples were immediately processed for microbiological and biophysical analyses.

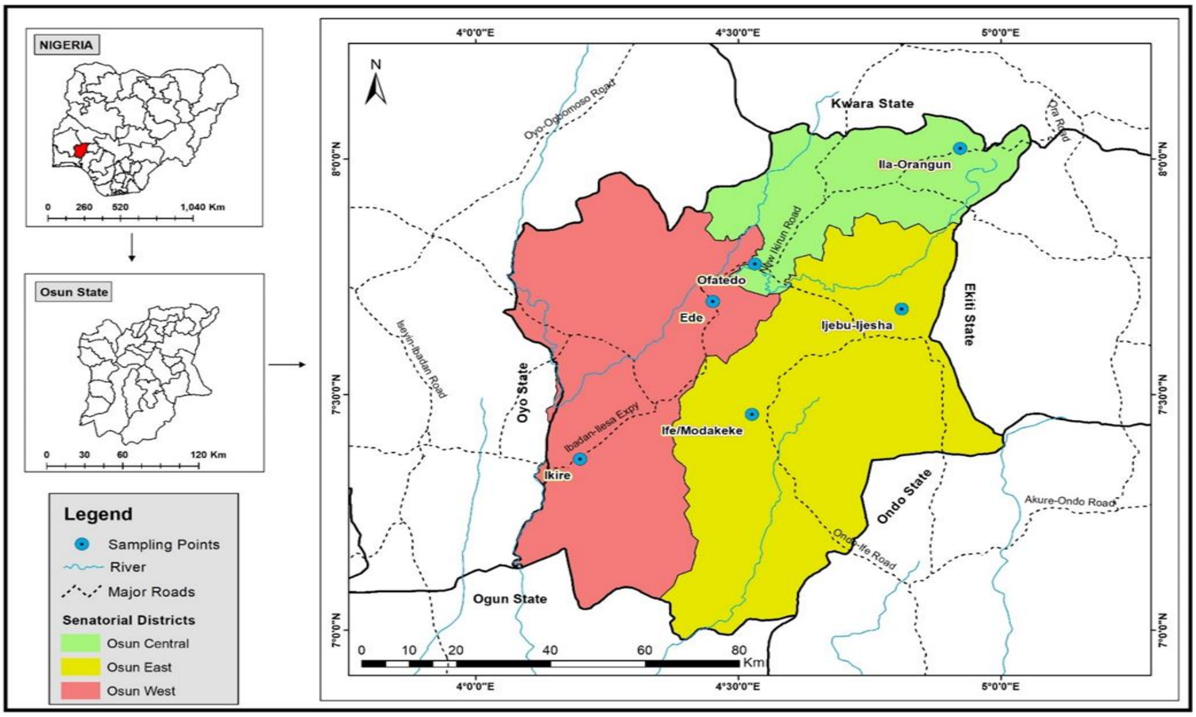


Figure 1: Map of Osun State showing sampled locations

Laboratory Processing of Samples

Serial dilution

Water samples were serially diluted to ensure manageable bacterial concentrations for enumeration. One milliliter of the water sample was aseptically transferred into nine milliliters of sterile distilled water using a sterile pipette. Four test tubes were prepared and labelled as 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}. From each dilution, 1 mL was transferred into sterile plates using the pour plate method for subsequent microbial analysis.

Media used for analysis

The study utilized various media for bacterial isolation and enumeration:

- Salmonella/Shigella Agar (SSA) and MacConkey Agar for isolating gram-negative bacteria.
- Eosin Methylene Blue (EMB) for identifying coliform bacteria.
- Nutrient Agar (NA) for general microbial growth.
- Mueller-Hinton Agar (MHA) for antibiotic susceptibility testing.

Plates were incubated for 24 hours at 37 °C following inoculation. Colony growth on the plates was sub-cultured onto fresh nutrient agar slants and then stored at 4 °C for further use.

Isolation on selective media

Distinct colonies from the initial plates were sub-cultured onto fresh nutrient agar plates using a sterile inoculating loop. Plates were streaked and then incubated for 24 hours at 37 °C to promote the growth of pure bacterial isolates. Slants were prepared in McCartney bottles for long-term storage, ensuring sterile conditions during the process.

Identification of Isolates

The colony morphology of the bacterial isolates was used to describe them, morphology, Gram staining reaction, and biochemical properties, following standard microbiological protocols. Further identification of isolates was performed using *Bergey's Manual of Systematic Bacteriology* (2019 edition), which provides a comprehensive framework for bacterial classification (Whitman et al., 2019).

Testing for antibiotic susceptibility

The disc diffusion method, which Bauer et al. (1966) created, was used to investigate the antibiotic susceptibility of bacterial isolates. Bacterial suspensions were spread on Mueller-Hinton agar plates, and antibiotic-impregnated discs were carefully placed on the surface. The plates were incubated at 37°C for a whole day. The surrounding zones of inhibition of the antibiotic discs were measured, and the results were interpreted according to the latest guidelines provided by the Clinical and Laboratory Standards Institute. (CLSI, 2023). The antibiotic discs included commonly used antibiotics in aquaculture and human medicine. Positive controls (susceptible bacterial strains) and negative controls (without antibiotics) ensured the reliability of the results.

Statistical analysis

Descriptive statistics, including mean and standard deviation, were used to summarize bacterial load, resistance patterns, and environmental factors. Cross-tabulation was employed to identify relationships between bacterial isolates and resistance patterns. Inferential statistics, such as chi-square tests, assessed associations between categorical variables, with significance set at $p < 0.05$. An SPSS software (version 17) facilitated data analysis.

Environmental controls

During the study, environmental factors such as pond type, water source, and feed type were recorded and controlled to minimize variability. The uniformity of sampling protocols ensured that environmental influences on microbial growth and resistance profiles were consistent across the sampling sites.

Results

The results of the study highlighted significant variations in microbial loads and the presence of bacteria across the sampled fish ponds. Figures 2, 3, and 4 provide visual representations of the study's findings. The observed trends indicate certain bacterial loads or frequencies across specific locations, expressing concerns regarding environmental and human health risks associated with the fish ponds. In fish pond water samples, microbial loads varied significantly among different locations, as shown in Figure 2. For *E. coli*, counts ranged from 1,600 to 5,200 CFU/ mL, with sample site D showing the highest load (5,200 CFU/mL) and site F the lowest (1,800 CFU/mL). *Klebsiella spp.* displayed broader variations, ranging from 14,100 CFU/mL at site B to 44,000 CFU/mL at site C. *Salmonella spp.*, on the other hand, exhibited relatively stable counts across sites, ranging from 400 CFU/mL at site E to 900 CFU/mL at site A. Statistical analysis using ANOVA confirmed significant differences (p = 6.66954E-06 < 0.05) among bacterial species across sampling locations.

In sediment samples (Figure 3), similar patterns of variability were observed. For *E. coli*, mean counts ranged from 1.60E+03 CFU/mL at site B to 2.09E+04 CFU/mL at site A. *Klebsiella spp.* had mean between 7.40E+03 CFU/mL at site F and 9.12E+04 CFU/mL at site A.

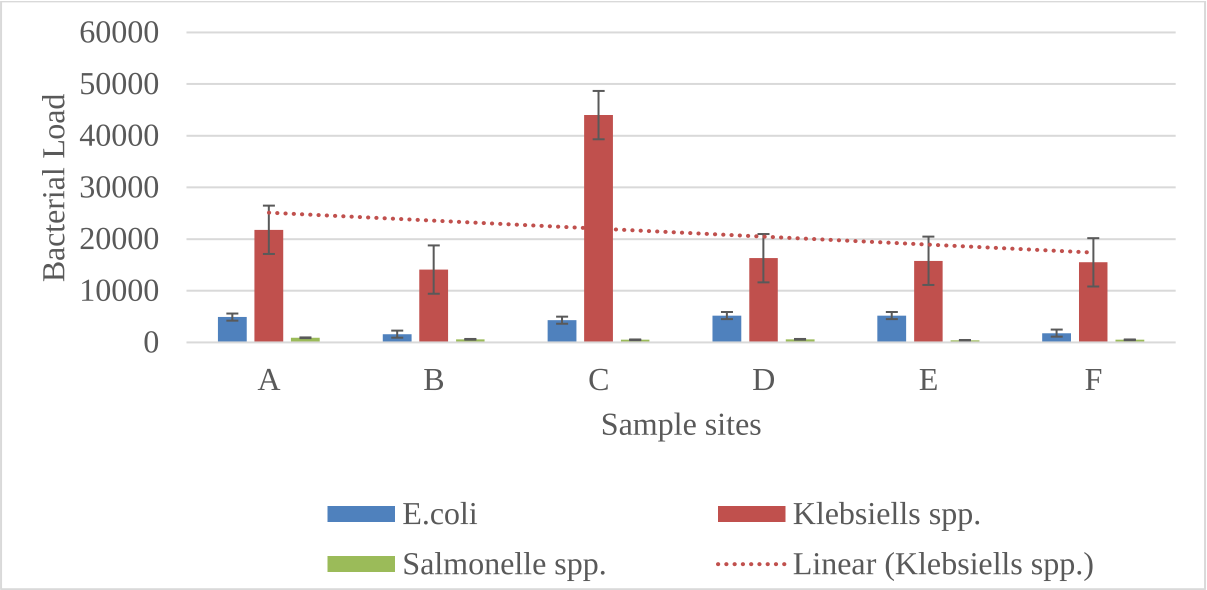


Figure 2: Bacteria load distribution in fish pond water sample in selected local government areas (LGAs) in Osun State. Key: A (Ife East LGA), B (Oriade LGA), C (Ede South LGA), D (Irewole LGA), E (Olorunda LGA), F (Ila LGA)

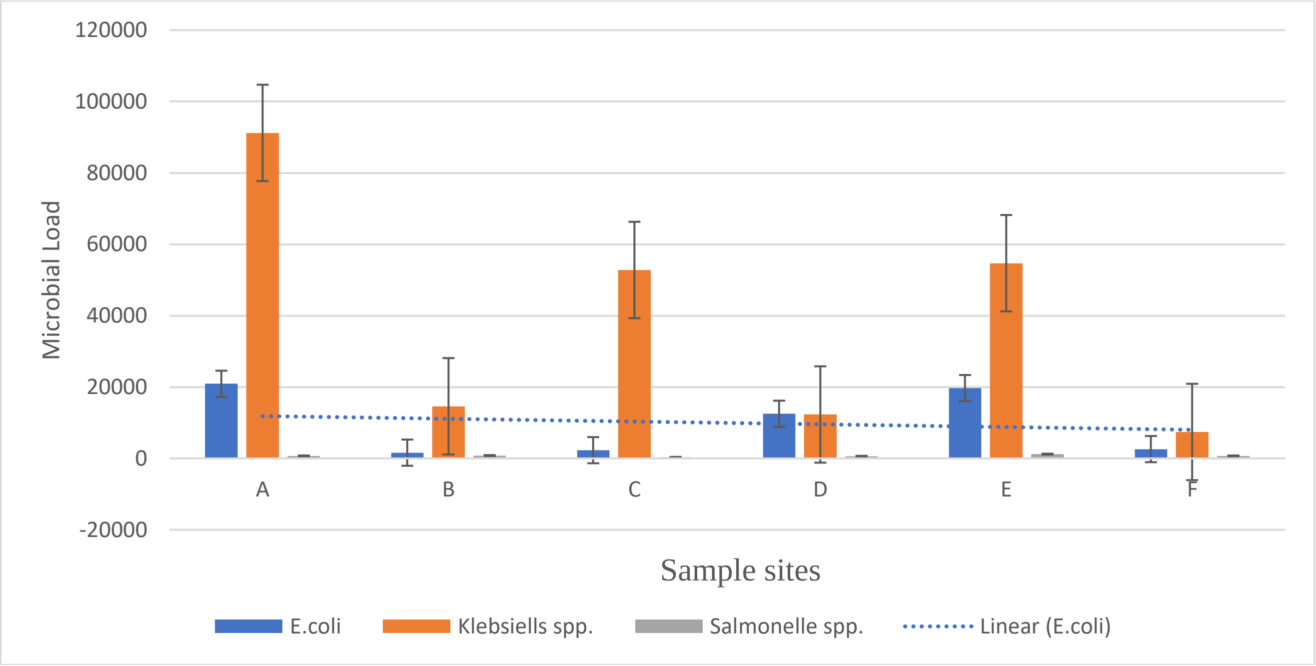


Figure 3: Bacteria load distribution in fish pond sediment samples in selected local government areas (LGAs) in Osun State. Key: A (Ife East LGA), B (Oriade LGA), C (Ede South LGA), D (Irewole LGA), E (Olorunda LGA), F (Ila LGA)

Salmonella spp. counts ranged from 3.00E+02 CFU/mL at site C to 1.20E+03 CFU/mL at site E. ANOVA indicated statistically significant differences in microbial loads among these bacteria, with a p-value of 0.002464 < 0.05. These variations highlighted site-specific factors influencing bacterial loads, such as environmental conditions and management practices, because of the disparities in the bacterial loads across the sampled ponds and locations.

The bacterial isolates from water and sediment samples displayed a wide range of diversity, as determined through macroscopic and microscopic examinations. Characterization was based on colonial morphology, Gram staining reaction, and biochemical tests, following standard microbiological procedures (Cheesbrough, 2006; Forbes et al., 2002). The analysis identified eleven genera of Gram-negative bacteria, including *Escherichia coli*, *Klebsiella* spp., *Salmonella* spp., *Proteus* spp., *Pseudomonas* spp., *Enterobacter* spp., *Citrobacter* spp., *Serratia* spp., *Shigella* spp., *Vibrio* spp., and *Providencia* spp.

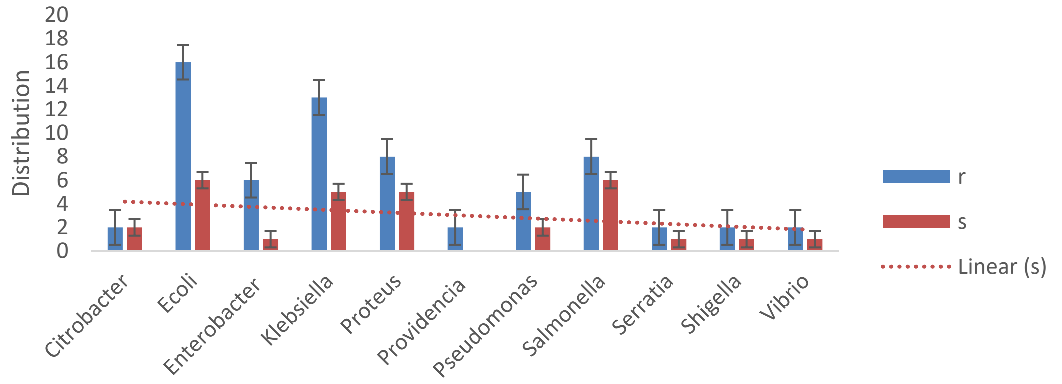
The frequency of bacterial isolates varied significantly across sites, as shown in Table 1. *E. coli* was the most prevalent, with 22 isolates (22.92%) identified across the sample sites. *Klebsiella spp.* followed with 18 isolates (18.75%), also present at all sites. *Salmonella spp.* was detected at five out of six locations, accounting for 14 isolates (14.58%). Other bacteria, such as *Proteus spp.* and *Pseudomonas spp.*, were found in moderate frequencies, with 13 isolates (13.54%) and seven isolates (7.29%), respectively. Less frequent species, including *Enterobacter spp.*, *Vibrio spp.*, and *Citrobacter spp.*, ranged from two to seven isolates (2.08 to 7.29%). These findings underscored the variability in bacterial composition and abundance in fish pond ecosystems, emphasizing the need for proactive management strategies to mitigate risks associated with these microorganisms in aquaculture environments.

Based on the findings shown in Figure 4, the antibiotic susceptibility (s) and resistance (r) patterns of bacterial isolates varied significantly across different species and antibiotics. The bacteria with the lowest “r” to Cefotaxime included *Shigella spp.*, *Vibrio spp.*, *Citrobacter spp.*, *Providencia spp.*, and *Serratia spp*, while higher “r” was exhibited by *Escherichia coli*, followed by *Klebsiella spp.*, *Proteus spp.*, and *Salmonella spp.* Tetracycline demonstrated high efficacy, with 90% of the bacterial isolates showing “s;” the least susceptible organisms to Tetracycline were *Vibrio spp.*, while *Klebsiella spp.* exhibited the highest “r”, followed by *E. coli*, *Enterobacter spp.*, *Shigella spp.*, and *Serratia spp.* For Ceftazidime, *Proteus spp.*, *Salmonella spp.*, *Klebsiella spp.*, and *E. coli* displayed the greatest “r”, whereas *Providencia spp.* and *Serratia spp.* exhibited the lowest levels. *E. coli* showed a higher “s” to Cotrimoxazole than *Klebsiella spp.*, *Salmonella spp.*, and *Proteus spp.*, with the lowest “s” observed in *Vibrio spp.* However, specific strains of *Klebsiella spp.*, *Enterobacter spp.*, and *Providencia spp.* demonstrated no “r” to Cotrimoxazole.

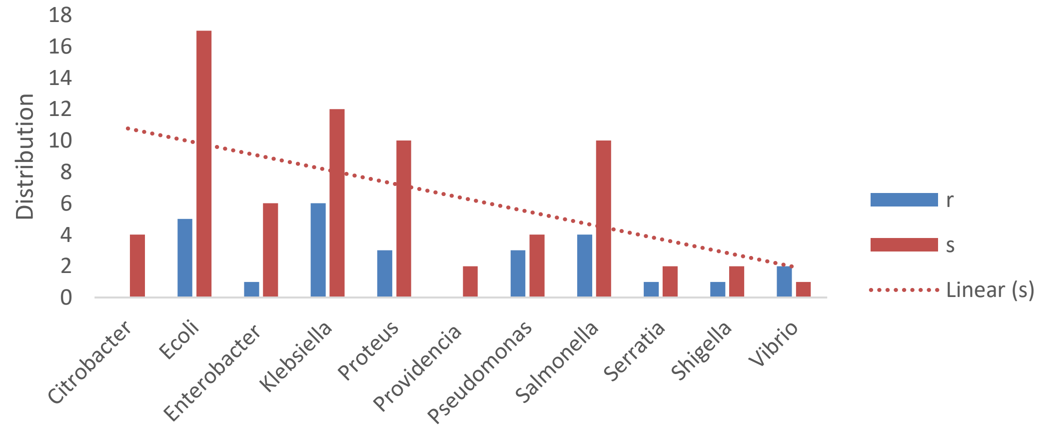
Gentamicin “r” was highest in *E. coli*, followed by *Klebsiella spp.* and *Salmonella spp.*, although *Klebsiella spp.* also displayed the highest “s” to this antibiotic. For Cefuroxime, *E. coli* was the most susceptible, followed by *Salmonella spp.* and *Klebsiella spp.*, with the least “s” observed in *Serratia spp.* Conversely, *Klebsiella spp.* showed the highest “r” to Cefuroxime, followed by *E. coli*, with the lowest “r” observed in *Vibrio spp.* The “s” patterns to Chloramphenicol showed that *E. coli* was the most susceptible, followed by *Salmonella spp.* and *Klebsiella spp.* The least susceptible organisms were *Providencia spp.* and *Serratia spp.* However, the bacteria with the highest “r” to Chloramphenicol were *Klebsiella spp.*, *E. coli*, and *Vibrio spp.* *Ceftriaxone* exhibited high efficacy against *E. coli*, which had the highest “s”, followed by *Klebsiella spp.* and *Proteus spp.*, with *Vibrio spp.* showing the least “s”. However, *Salmonella spp.* displayed the highest “r” to Ceftriaxone, followed by *Klebsiella spp.*, with the lowest “r” observed in *Providencia spp.* and *Serratia spp.* The *E. coli* has the highest susceptible pattern against Ciprofloxacin, followed by *Klebsiella spp, Proteus spp and Salmonella spp*. and the least was in *Vibrio spp*. Although *Salmonella spp* still showed the highest "r" against Ciprofloxacin but the least was in *Citrobacter spp*. The "r" of all the isolated bacteria to Vancomycin was evident, where *E. coli* had the strongest pattern, then by *Klebsiella spp*. and *Shigella spp*. with the lowest.

Table 1: Frequency of bacteria isolated from the fish pond water and fish pond sediment samples

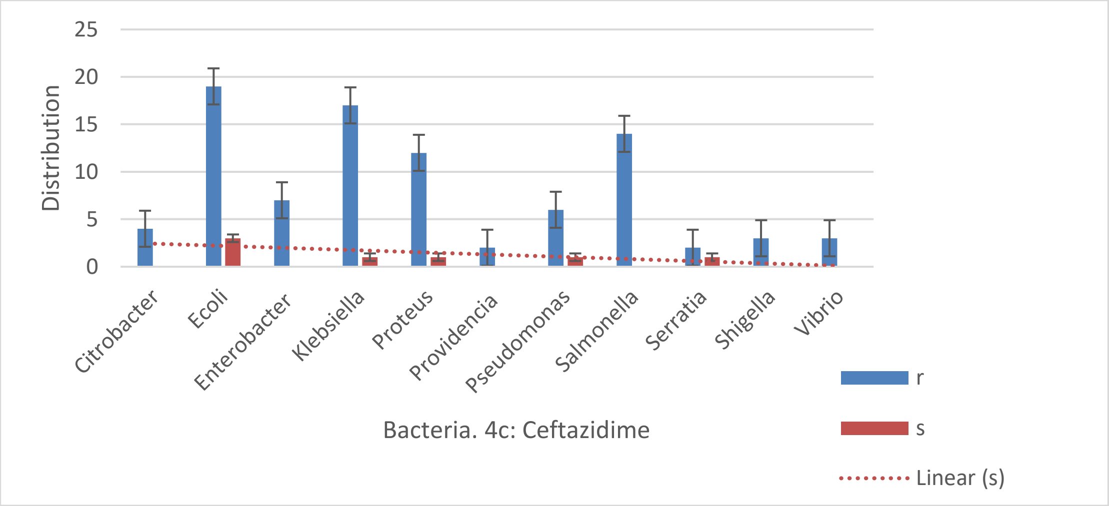
Sampled site Bacteria	1	2	3	4	5	6	Frequency	% Frequency
Vibrio spp	+	-	-	+	-	-	3	2.88
Citrobacter spp	-	-	-	+	+	-	4	3.84
E. coli	+	+	+	+	+	+	22	22.92
Enterobacter spp	+	-	+	-	-	-	7	7.29
Klebsiella spp	+	+	+	+	+	+	18	18.75
Proteus spp	+	+	-	-	+	-	13	13.54
Providencia spp	-	-	-	+	-	+	2	2.08
Pseudomonas spp	+	+	-	-	-	-	7	7.29
Salmonella spp	+	+	-	+	-	+	14	14.58
Serratia spp	-	+	+	-	-	-	3	3.13
Shigella spp	-	-	+	+	-	+	3	3.13



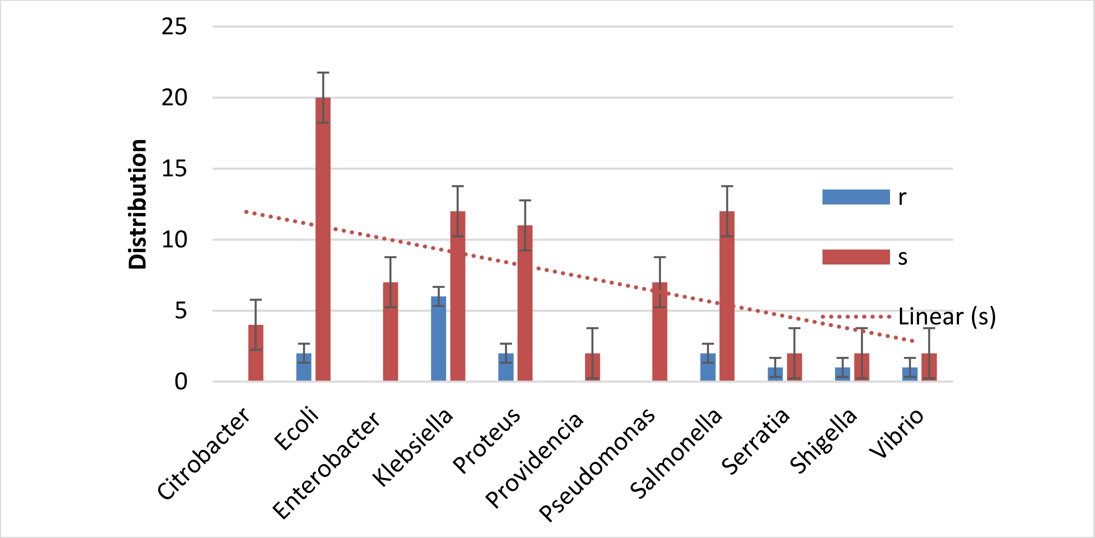
Bacteria. 4a Cefotaxime



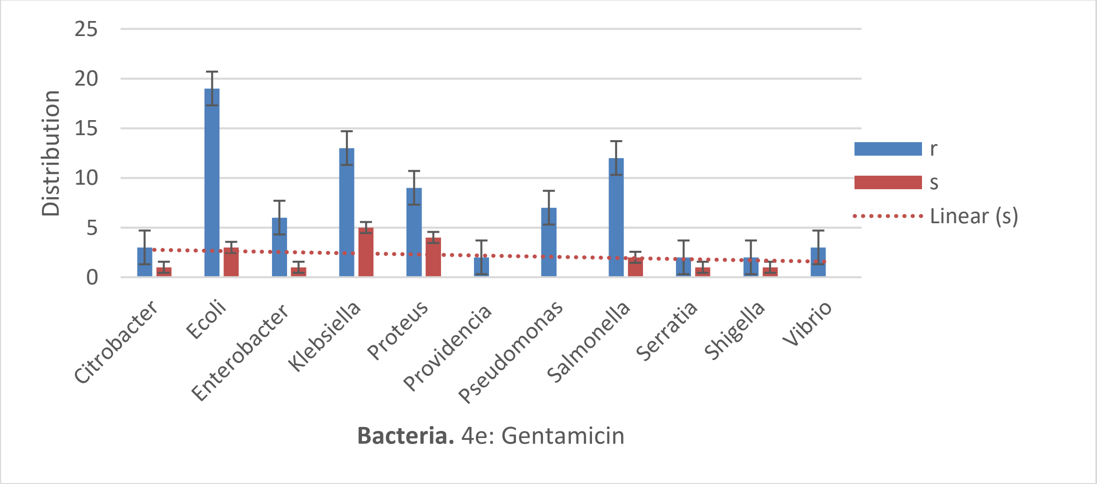
Bacteria. 4b: Tetracycline



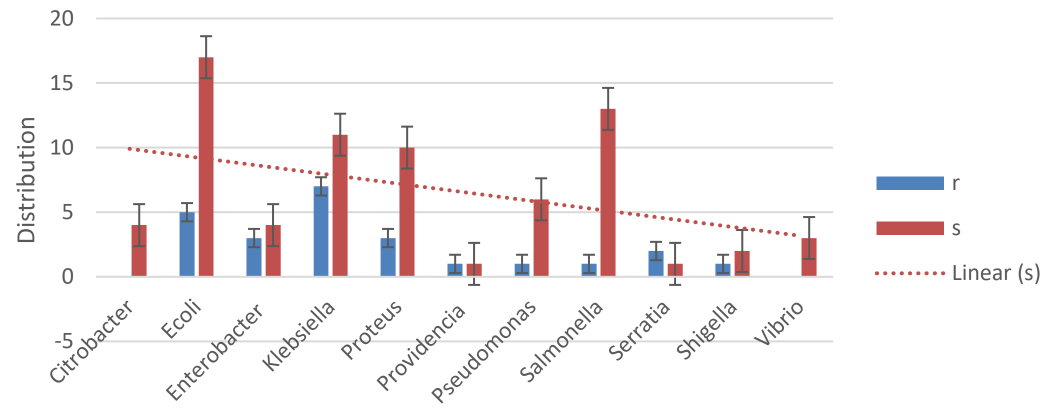
Bacteria. 4c: Ceftazidime



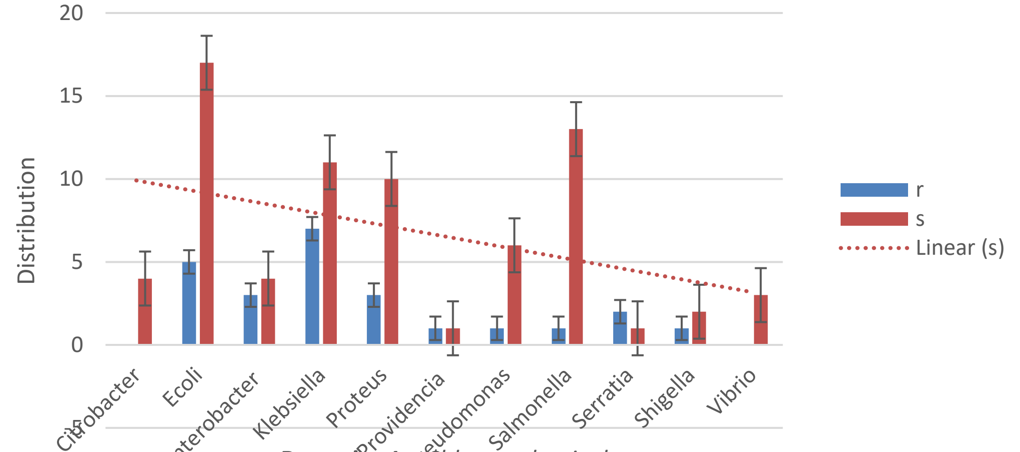
4d: Cotrimoxazole



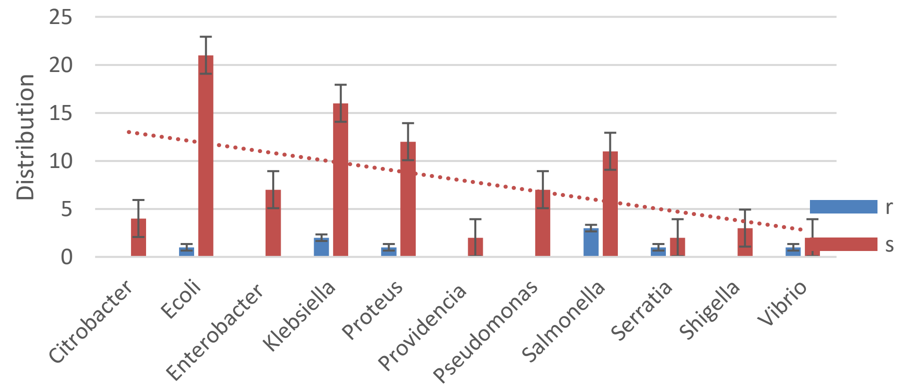
Bacteria. 4e: Gentamicin



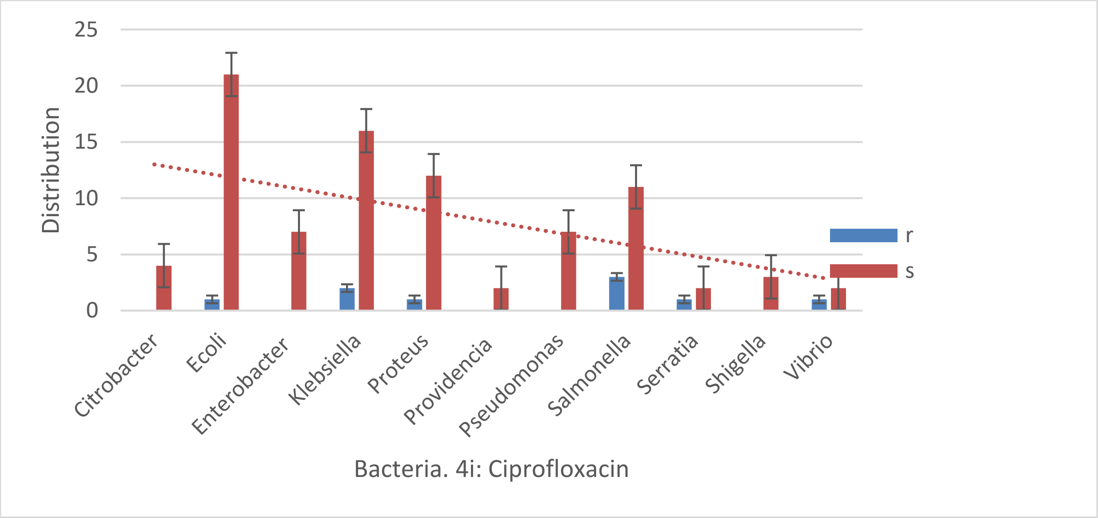
Bacteria. 4f: Cefuroxime

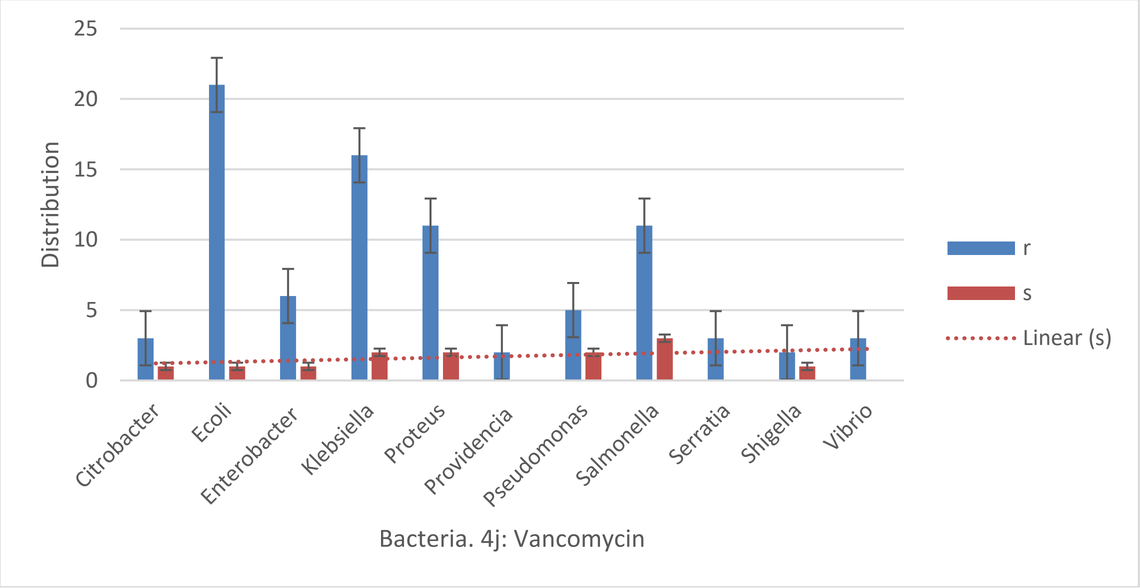


Bacteria. *4g: Chloramphenicol*



Bacteria. 4h: Ceftriaxone





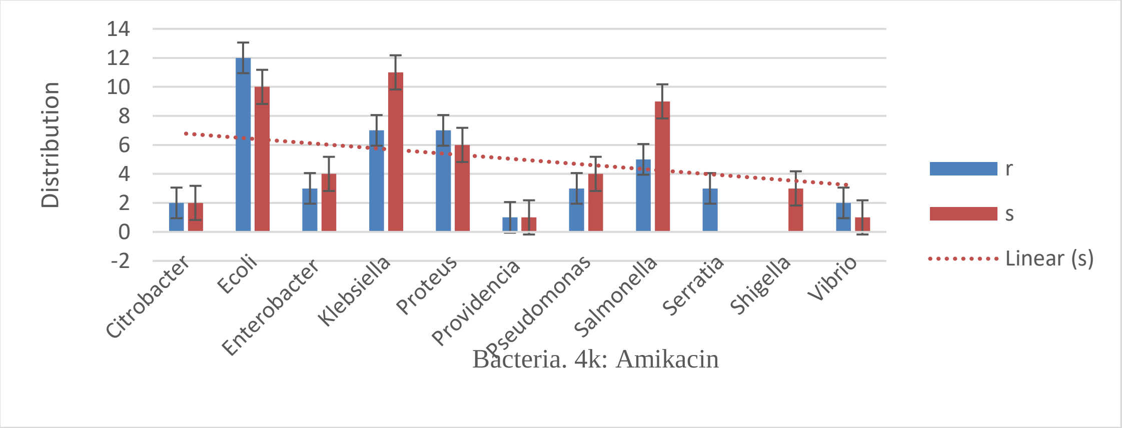


Figure 4(a-k): Susceptibility pattern of the isolated bacteria against antibiotics

However, *Salmonella* species showed the highest “s” to Vancomycin, whereas *Vibrio* species showed the lowest. *Escherichia coli* has the highest “r” pattern against Amikacin, followed by *Klebsiella spp* and *Proteus spp*., and the least “r” was recorded for *Providencia spp,* while *Klebsiella spp* showed the highest “s” and least was in *Seratia spp*. The analysis of antibiotic susceptibility revealed significant f presence of multidrug-resistant (MDR) bacteria among enterobacteria isolates to three or more antibiotics. In fish pond water (Table 2), *Escherichia coli* exhibited the highest prevalence of MDR phenotypes, showing resistance to four antibiotics; Glycopeptides, Tetracyclines, Aminoglycosides, and Cephalosporins. *Salmonella spp.* resisted Glycopeptides, Aminoglycosides, and Cephalosporins. In contrast, *Enterobacter spp., Klebsiella spp., and Proteus spp*. resisted Glycopeptides and Cephalosporins. *Citrobacter spp., Pseudomonas spp., and Serratia spp*. did not exhibit any MDR phenotypes.

Thus, only *E. coli* and *Salmonella spp*. were identified as typical MDR bacteria in the fish pond water. In the fish pond sediment (Table 2), *Klebsiella spp*. displayed the highest MDR phenotypes, showing resistance to six antibiotics; Glycopeptides, Aminoglycosides, Tetracyclines, Macrolides, Sulfonamides, and Cephalosporins. *Escherichia coli* exhibited resistance to four antibiotics, including Glycopeptides, Aminoglycosides, Macrolides, and Cephalosporins. Meanwhile, *Proteus spp., Pseudomonas spp., and Salmonella spp*. showed resistance to Glycopeptides, Aminoglycosides, and Cephalosporins. However, *Citrobacter spp., Enterobacter spp., Providencia spp., Serratia spp., Shigella spp., and Vibrio spp*. isolated from the sediment did not exhibit MDR phenotypes.

Discussion

Bacterial contamination in fish ponds

The study investigates significant bacterial contamination across selected fish ponds, highlighting high bacterial loads and total coliform counts to exceed World Health Organization (WHO, 2020) standards; this reflects poor water quality for serious public health risks. Contamination is primarily linked to anthropogenic activities, including inadequate waste management, agricultural runoff, and poor fish pond maintenance. Similar findings by Kariuki et al. (2019) and Jaramillo et al. (2021) confirmed that such activities are global contributors to bacterial contamination in aquaculture environments.

On the other hand, key factors driving contamination include fecal pollution, nutrient-rich sediments, and organic waste accumulation, which provide an ideal environment for bacterial growth. Hu et al. (2020) and Zhao et al. (2022) also associated water contamination with improper land use and sewage discharge.

Pathogenic bacteria in aquaculture systems

The identification of *Escherichia coli*, *Salmonella* spp., *Klebsiella* spp., and other pathogens underscores the risks aquaculture poses to aquatic ecosystems and human health. Persistent pathogens, including *Vibrio* spp., *Aeromonas* spp., and *Salmonella* spp., have been associated with direct contamination through human and animal activities. Research by Chen et al. (2021) and Lopes et al. (2022) similarly highlighted the prevalence of these pathogens in aquaculture environments, exacerbated by poor hygiene and waste disposal practices.

The high prevalence of *E. coli* in this study aligns with findings by Wang et al. (2021), linking its dominance to wastewater mismanagement and agricultural runoff. Prominent isolation of *Klebsiella* spp. and *Salmonella* spp. emphasizes faecal matter contamination and inadequate hygiene practices, corroborated by studies from Musa et al. (2021) and Acharya et al. (2022).

Table 2: Antibiotic resistant pattern of isolated bacteria from fish pond water and sediment

Classes of Antibiotics Organism in **water**	Glycopeptides	Aminoglycosides		Tetracycline	Macrolides	Quinolones	Sulfonamides	Cephalosporin				Multi-drug resistant
Antibiotics	VAN	AMK	GEN	TET	CHL	CIP	COT	CTX	CPZ	CRX	CTR	
Citrobacter spp		-	-	-	-	-	-	1	1	1	-	0
E. coli	8	3	1	3	2	-	1	6	7	8	8	4
Enterobacter spp	4	2	1	-	1	-	-	3	4	3	3	2
Klebsiella spp	4	1	2	1	1	-	-	2	5	2	3	2
Proteus spp	3	2	1	1	1	-	1	1	3	2	3	2
Pseudomonas spp	1	1	-	-	1	-	-	-	-	1	-	0
Salmonella spp	5	3	-	1	1	1	-	4	6	5	3	3
Serratia spp	1	1	-	-	-	-	-	-	-	-	1	0

Key: Gentamicin (GEN), Tetracycline (TET), Ceftazidime (CPZ), Cefuroxime (CRX), Ciprofloxacin (CIP), Amikacin (AMK), Vancomycin (VAN), Chloramphenicol (CHL), Cotrimoxazole (COT), Ceftriaxone (CTR) and Cefotaxime (CTX)

Classes of antibiotics Organism in **sediment**	Glycopeptides	Aminoglycosides		Tetracycline	Macrolides	Quinolones	Sulfonamides	Cephalosporin				Multi-drug-resistant
	VAN	AMK	GEN	TET	CHL	CIP	COT	CTX	CPZ	CRX	CTR	
Citrobacter spp	3	2		-	-	-	-	1	3	2		2
E. Coli	13	9	2	2	3	1	1	10	12	11	7	4
Enterobacter spp	2	1	1	1	2	-	-	3	3	3	3	1
Klebsiella spp	12	6	3	5	6	2	6	11	12	11	10	6
Proteus spp	8	5	1	2	2	1	1	7	9	7	6	3
Providencia spp	2	1	-	-	1	-	-	2	2	2	2	0
Pseudomonas spp	4	2	2	3	-	-	-	5	6	6	5	3
Salmonella spp	6	2	1	3	-	2	2	4	8	7	3	3
Serratia spp	2	2	1	1	2	1	1	2	2	2	2	1
Shigella spp	2			1	1	-	1	2	3	2		1
Vibrio spp	3	2	1	2	-	1	1	2	3	3	2	2

Antibiotic resistance

The study also determines alarming antibiotic resistance trends among bacterial isolates. The resistance of *E. coli*, *Klebsiella* spp., *Proteus* spp., and *Salmonella* spp. to commonly used antibiotics such as cefotaxime, gentamicin, and ciprofloxacin highlights the consequences of unregulated antibiotic use in aquaculture; these findings are consistent with those of Faruk et al. (2021) and Li et al. (2020), who attributed resistance to selective pressure from extensive antibiotic exposure and horizontal gene transfer in aquatic environments. Although tetracycline remains effective against many bacterial strains, its overuse raises concerns about potential resistance, as noted by Singh et al. (2021). Similarly, misuse of fluoroquinolones like ciprofloxacin has contributed to resistant strains, consistent with findings by Amuguni et al. (2022) and Zhang et al. (2023).

Multiple antibiotic-resistant patterns of isolated bacteria

The rise of *Escherichia coli* (*E. coli*) as a multidrug-resistant (MDR) bacterium in fish pond environment demonstrates resistance to glycopeptides, tetracyclines, aminoglycosides, and cephalosporins, and poses a significant public health threat. According to Cabello et al. (2016), the excessive and indiscriminate use of antibiotics in aquaculture is a major factor driving the development of multidrug resistance in *E. coli*. Antibiotics are widely used to treat, prevent, and promote the growth of bacterial infections, which creates selective pressure that helps resistant forms of bacteria survive and proliferate. Prolonged exposure to sub-inhibitory concentrations of antibiotics allows *E. coli* to develop resistance through a combination of horizontal gene transfer and intrinsic mechanisms (Baquero et al., 2021). One of the critical factors contributing to MDR *E. coli* in fish pond environments is the accumulation of antibiotic residues, which enter aquatic systems through direct application in aquaculture, runoff from agricultural activities, and improper disposal of pharmaceutical waste (Kümmerer et al., 2018). Persistent residues across the sediment and water samples provide a continuous source of selective pressure, accelerating the development of resistance in bacterial population. Kim et al. (2021) highlighted that *E. coli* can adapt to environmental stressors by acquiring resistance genes through plasmids, integrons, and transposons, further enhancing its adaptability and resistance potential. The implications of MDR *E. coli* extend beyond aquaculture, with potential risks to human health due to the transmission of resistant bacteria through water, fish, or environmental exposure. According to Shen et al. (2020), fish pond environment contaminated with MDR *E. coli* serves as reservoirs of resistance genes that can spread to human pathogens, exacerbating public health challenges. The findings underscored the urgent need for regulatory measures to control antibiotic use in aquaculture, improve wastewater management, and promote sustainable aquaculture practices to mitigate the spread of antibiotic resistance.

The emergence of *Salmonella* spp. as MDR pathogens in aquaculture, exhibiting resistance to multiple antibiotic classes—such as glycopeptides, aminoglycosides, and cephalosporins—is a critical public health concern. The widespread, often unregulated use of antibiotics in fish farming has been identified as a leading cause of MDR in *Salmonella*. Antibiotics like cephalosporins and aminoglycosides are commonly administered for growth promotion and disease prevention, frequently without veterinary oversight or proper dosage control, contributing to resistance development (Cabello et al., 2016); this selective pressure enables resistant strains of *Salmonella* to proliferate and dominate in fish pond environment. In aquaculture systems with high bacterial densities, horizontal gene transfer (HGT) serves as a major mechanism for the dissemination of resistance genes among bacterial populations. Studies indicate that HGT plays a significant role in the spread of resistance genes across diverse bacterial species in aquatic environments (Mather et al., 2018). The capacity of *Salmonella* spp. to develop resistance to various antibiotics, including glycopeptides and cephalosporins, even in the absence of direct exposure, underscores their resilience and adaptability (Martinez et al., 2019).

The development of MDR *Salmonella* spp. is often driven by the accumulation of antibiotic residues in aquaculture systems. Residues from sources such as agricultural runoff, untreated wastewater, and improper disposal of pharmaceutical products serve as reservoirs for resistance genes. These residues can persist in sediment and water, creating a sustained selective environment that promotes the emergence of resistant strains (Hu et al., 2019). The health implications of MDR *Salmonella* in fish ponds are far-reaching. Humans consuming fish or seafood contaminated with MDR *Salmonella* face a heightened risk of severe infections, including gastroenteritis. The resistance of *Salmonella* spp. to multiple antibiotics complicates treatment options, often resulting in prolonged illness, increased healthcare costs, and higher risks of complications (Threlfall, 2002). The detection of *Klebsiella* spp. as MDR pathogens in fish pond sediments, with resistance to six antibiotics—glycopeptides, aminoglycosides, tetracyclines, macrolides, sulfonamides, and cephalosporins—highlights the growing challenge of antibiotic resistance in aquaculture. These bacteria often associate with environments impacted by poor waste management and agricultural runoff, which contribute to high levels of antibiotic-resistant bacteria in aquatic systems (Kümmerer et al., 2018).

Sediments act as reservouirs for MDR bacteria, enabling their persistence and proliferation. Resistant pathogens from sediments can enter the water column, affecting fish health and contaminating the food chain (Rico et al., 2013). Organic matter accumulation in pond sediments provides an ideal reservoir for pathogens, as observed by Tang et al. (2021) and Qiu et al. (2023); this promotes the persistence of resistant strains, which can cause gastrointestinal infections in humans and aquatic species. The accumulation of antibiotic-resistant *Klebsiella* spp. in aquaculture systems not only poses risks to human health but also threatens the sustainability of fish farming practices. Effective waste management and stricter regulations on antibiotic usage are essential to mitigate these risks.

Conclusion and Recommendations

The study highlights the pervasive presence of pathogenic and antibiotic-resistant bacterial species profiles in fish pond water and sediment, emphasizing the urgent need for improved aquaculture management practices. Issues such as fecal contamination, agricultural runoff, inadequate waste management, and misuse of antibiotics compromise the quality of fish pond water, posing significant risks to aquatic ecosystems and public health. The findings underscore the intricate relationship between anthropogenic activities, environmental factors, and the emergence of antibiotic resistance, necessitating stringent monitoring and regulatory measures to safeguard water quality and public health. It has also unequivocally revealed the alarming presence of multidrug-resistant (*MDR*) bacteria, including *Pseudomonas*, *Proteus*, *Salmonella*, *Klebsiella*, and *Escherichia coli*, in fish pond water and sediment, raising significant concerns about the potential implications for human health, animal health, and the environment.

The following measures are recommended to ensure public safety and promote sustainable aquaculture practices.

Proper construction and maintenance of fish ponds: Fish ponds should be strategically constructed to minimize exposure to pollutants and weeds that can introduce harmful microorganisms. Ponds should be designed to prevent contamination from passive processes like wind and rainfall.

Regulatory standards and enforcement: Among other regulatory agencies is the National Agency for Food and Drug Administration and Control (NAFDAC) should establish and enforce stringent guidelines for the aquaculture industry in Nigeria. Compliance with such standards will not only enhance local fish farming practices but also improve the potential for exporting fish stocks to international markets.

Improvement of sanitary conditions in fish farms: Adopt good aquaculture practices, including the use of high-quality water and feeds with minimal microbial contamination. Implement regular pond water draining schedules and restrict public access to fish farms to prevent contamination.

Enhanced wastewater management: Establish efficient wastewater management systems within fish farms collect, treat, and adequately dispose of wastewater, reducing the risk of contamination.

Professional implications of the study

Antibiotic resistance can increase the risk of disease transmission, serious sickness, disability, and death by making infections difficult or impossible to cure. Additionally, resistant bacteria can prolong hospital stays for patients and compromise successful health outcomes.

Environmental and public health implications

The presence of antibiotic-resistant bacteria in aquaculture environments poses significant threats to aquatic ecosystems, human health, and food safety. Mitigating these challenges requires improved aquaculture management practices, strict regulation of antibiotic use, and regular water quality monitoring. Reduction of organic waste inputs and enhanced waste management strategies are essential for minimizing bacterial contamination and safeguarding public and environmental health.

Limitations to the study

The findings of this study are confined to fish ponds in Osun State, Nigeria and may not be extrapolated to other places. It employed traditional microbiological culture methods, which may not be able to identify some bacterial species or patterns of resistance. The role of environmental and anthropogenic variables in the development of resistance was not studied in a deeper level.

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Informed consent statement

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Conflicts of interest

The authors declare no conflict of interest.

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